Standard dilution analysis in flow system: Sodium determination by flame atomic emission spectrometry

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1. Introduction

In analytical chemistry, instrumental methods generally require careful calibration for determining the concentration of an analyte in an unknown sample [1,2]. External standard calibration (ES) is the most commonly used calibration method due to its simplicity of application and interpretation [3]. However, this method is susceptible to errors mainly caused by variations in instrumental parameters and/or matrix effects which can deteriorate precision and/or accuracy. These drawbacks can be circumvented by using other types of calibration such as matrix matching, standard addition (SA) and internal standardization (IS) [4–6].

A novel calibration method called standard dilution analysis (SDA), that combines the principles of the traditional methods of SA and IS was recently proposed in the literature [7]. It can be performed by providing the detector with a solution containing a constant amount of sample and a varying amount of standard solution containing both analyte and internal standard. SDA was applied to sodium determination in biodiesel samples and certified reference materials (CRMs) by flame atomic emission spectrometry (FAES). Lithium was employed as internal standard in all determinations. The results for Na determination in five CRMs using SDA were in agreement with certified values at the 95% confidence level (t-test). For comparison purposes, Na was also determined by the traditional methods of external standard (ES), standard additions (SA) and internal standardization (IS). Recoveries showed increased accuracy following the sequence ES (181–202%) < IS (67–72%) < SA (111–126%) < FIA-SDA (94–98%). FIA-SDA provided more accurate and precise results than ES, SA and IS for Na determination in biodiesel and CRMs by FAES.

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2. Materials and methods

2.1. Reagents, analytical solutions and samples

High purity deionized water obtained using a Millipore Rios 5® reverse osmosis and a Millipore Milli-Q® Academic® deionizer system (resistivity 18.2 MΩ cm, Millipore, Bedford, USA) was used to prepare all solutions.

Analytical solutions were prepared by appropriate dilution of 1000 mg L\(^{-1}\) Na and Li stock solutions (SpecSol, São Paulo, Brazil). Distilled ethanol was used for biodiesel samples dilution. Nitric acid 70% (JT Baker, Phillipsburg, USA) and H\(_2\)O\(_2\) 30% v v\(^{-1}\) (Merck, Darmstadt, Germany) were used for CRMs digestion.

All solutions were stored in high-density polypropylene flasks (Nalgene, Rochester, USA). All plastic containers and glassware materials were decontaminated by soaking in 10% (v v\(^{-1}\)) H\(_2\)O\(_2\) for at least 24 h, and then rinsing abundantly in deionized water before use.

Biodiesel samples provided by the Monitoring and Research Center of Fuel Quality, Biofuels, Oil and Derivatives (CEMPEQC, Araraquara, Brazil) were stored in amber glass flasks followed by stored in a refrigerator (about 5 °C) before analysis. A mass of 2.0 g biodiesel samples and 2.0 g biodiesel standard oil (Conostan, Baie-D’Urfé, Canada) were directly weighed into 15-mL polypropylene graduated cylinders (Corning, New York, USA) and the volumes were made up to 10 mL with distilled ethanol.[10,11]

All CRMs (1549 Non-Fat Milk Powder, 8435 Whole Milk Powder, 1577b Bovine Liver, and 2976 Mussel Tissue) from the National Institute of Standards and Technology (Gaithersburg, USA) were prepared by microwave-assisted wet digestion. Sample masses of ca. 200 mg were accurately weighed and transferred to microwave flasks followed by 3.0 mL H\(_2\)O\(_2\), 1.0 mL H\(_2\)N\(_2\)O\(_3\), and 2.0 mL of deionized water. Then, the mixtures were heated using the following five-step microwave program: (1) 15 min from 0 to 600 W; (2) 5 min at 600 W; (3) 15 min from 600 to 800 W; (4) 5 min at 800 W and (5) 20 min at 0 W (cooling). After cooling, the digests were transferred to 25 mL volumetric flasks and diluted to the mark with deionized water.

2.2. Instrumentation

An Analyst 100 Perkin Elmer flame atomic absorption spectrometer (Shelton, USA) equipped with a 50-mm burner head was used for sample nebulization and analyte excitation. An air-acetylene flame was employed for Na and Li excitation. The optimum air-acetylene flow-rates for Na and Li excitation was a 4:2 (air:acetylene) ratio. Acetylene with 99.7% purity (Air Liquid, São Paulo, Brazil) was used as fuel gas.

The emission intensities from Na and Li were measured at 586.98 nm and 667.43 nm, respectively, using an US 650 Red Tide Ocean Optics spectrometer (Dunedin, FL, USA). The acquisition of emission signals by the Ocean Optics spectrometer (SpetraSuite software) employed a 50-ms integration time in high-speed mode (6000 spectra recorded by each analytical cycle).

An IPC-8 Ismatec peristaltic pump (Zurich, Switzerland) furnished with Tygon® tubing was used for pumping solutions.

A Multiwave 3000 Anton Paar (Graz, Austria) microwave oven with a rotor of 48 reaction PFA vessels (internal volume of 50 mL) was used to digest all CRMs.

2.3. The flow system

The FIA-SDA system comprised a peristaltic pump, Tygon® pumping tubes, a manual injector-commutator[12], a flame atomizer, a fiberoptic spectrophotometer, polyethylene tubing (i.d. 0.8 mm), coiled reactor and accessories. The flow diagram of the simultaneous Na and Li signal acquisition system is shown in Fig. 1. The operation of the injector-commutator IC involves the sequential introduction of solutions A and B in the system. In the position specified in the figure, an aliquot of solution B (S\(_b\)) selected by the loop L\(_2\) is injected into the carrier water carrier stream (C). The established sample zone reaches the atomizer and a steady-state emission signal is measured. After 150 s, the IC is switched and the solution B (S\(_b\)) selected by the loop L\(_2\) is injected into the carrier C. Passage of the sample zone through the flame results in a steady-state signal. After baseline restoration, another cycle can be started. A typical signal profile obtained with the proposed flow system is depicted in Fig. 2.

2.4. Analytical procedure

The SDA method[7] requires just a blank and two analytical solutions (A and B) for each sample. Solution A is a mixture of standards (analyte plus internal standard) and sample (1:1 ratio by volume), and solution B is a mixture of sample and blank solutions (1:1 ratio by volume). The solutions must be simultaneously measured in order to obtain a typical SDA graph.[7] Considering the flow system in Fig. 1, the injected sample volume (length of the sampling loops) and the length of reactor coil were evaluated to ensure well-defined steady-state

Fig. 1. Flow diagram of the FIA-SDA system for sodium determination. IC: injector-commutator, L\(_1\), L\(_2\): sampling loops (2.5 mL), B\(_1\): coiled reactor (110 cm); C: blank solution (1.0 mL min\(^{-1}\)), S\(_a\): solution A (1.0 mL min\(^{-1}\)), S\(_b\): solution B (1.3 mL min\(^{-1}\)), PD: pulse dampener, W: wastes; CCD: detector (586.98 and 667.43 nm). The downward arrows indicate the movement of the central part of IC.
signals for \( S_A \) and \( S_B \), and a definite transition curve (SDA region) between them. In this study, solutions \( S_A \) and \( S_B \) were prepared by using 0.5 mg L\(^{-1}\) Na as sample solution, a standard solution containing 1.0 mg L\(^{-1}\) Na + 1.0 mg L\(^{-1}\) Li and deionized water as blank.

The SDA method requires the simultaneous collection of emission intensities of Na (\( I_{Na} \)) and Li (\( I_{Li} \)) at the transitional curve between steady-state regions (SDA curve), and the corresponding Li concentrations. Signal intensities and internal standard concentration (CIS) are employed to build SDA plots (\( I_{Na}/I_{Li} \) versus \( 1/[Li] \)) from which analyte concentration in the sample (\( C_{sam} \)) is calculated by using the following equation 
\[
C_{sam} = (\text{slope}/\text{intercept}) \times (C_{std}/C_{IS}),
\]
where \( C_{std} \) and \( C_{IS} \) are standard and internal standard concentrations in the originally prepared standard solution, respectively [7]. Lithium concentration was calculated at different points of the SDA region by linear interpolation of Li emission intensities within the baseline (zero concentration) and steady state signal (known Li concentration).

Considering the excessive number of spectra for calibration, the use of a set of 10, 20, 30, 40 and 50 points to generate an average spectrum was evaluated.

The influence of data acquisition at different sub-regions of the SDA region on accuracy was evaluated. Different sub-regions were selected in order to cover emission intensities from the steady-state (100% intensity) to baseline (0% intensity) interval: 100–0%; 90–10%; 90–0%; 100–10%.

Determination of Na in samples and CRMs by using comparative calibrations (ES, SA and IS) were performed in seven replicates in batch mode. The aspiration rate of the flame spectrometer was fixed to 5.0 ml min\(^{-1}\).

2.5. Sodium determination

After system optimization, the method was applied to the determination of Na in five biodiesel samples. Accuracy was checked by analyzing different CRMs. The performance of the procedure was also evaluated by comparing the FIA-SDA results with values obtained with ES, SA and IS.

The proposed procedure involves the preparation of two solutions (\( A \) and \( B \)) for biodiesel analysis: 50% sample solution + 50% standard (1.0 mg L\(^{-1}\) Li + 1.0 mg L\(^{-1}\) Na in ethanol); and 50% sample solution + 50% ethanol (blank). The SDA plots were constructed with the Na/Li emission intensity ratio on the y-axis versus \( 1/[Li] \) on the x-axis. For analysis of milk, bovine liver and mussel tissue CRMs, ethanol was replaced by water as solvent.

Fig. 3. Influence of sample loop volume. Signals for Na and Li were obtained with the system in Fig. 1 using \( L_1 \) and \( L_2 = 0.5 \) ml (a), 1.0 ml (b), 1.5 ml (c) and 2.5 ml (d).
External standard calibration curves were built up by plotting INa versus [Na] for analytical solutions in the 0.1–1.0 mg L\(^{-1}\) range.

For IS calibration, all blanks, analytical solutions (0.1–1.0 mg L\(^{-1}\) Na) and samples were spiked with 0.5 mg L\(^{-1}\) Li. Calibration curves were built up by plotting INa/ILi versus [Na].

The SA calibration curves were built up by plotting INa versus [Na] for spikes at final concentrations of 0.0 ("unspiked"), 0.1, 0.2, 0.3, 0.4 and 0.5 mg L\(^{-1}\) Na in 10% (m v\(^{-1}\)) of biodiesel.

Accuracy and precision were also evaluated by means of addition and recovery tests for biodiesel samples spiked with 0.2 mg L\(^{-1}\) Na.

3. Results and discussion

3.1. Optimization of the flow system

The flow system depicted in Fig. 1 was designed to obtain limited dispersion [13]. Initially, the coiled tube (B1) was 110 cm, the shortest length of tubing connecting the IC to the flame of spectrometer, avoiding a rigid arrangement of the flow setup. The flow-rate of the carrier stream C was fixed at 1.0 mL min\(^{-1}\) as a good compromise among analytical frequency, overpressure and establishment of a well-defined SDA region. Considering that the nebulization chamber of the flame spectrometer may act as a diluting chamber [14,15], and that limited dispersion can be obtained in single-channel systems by increasing the sampling loop [13], the influence of the injected sample volume on signal profiles was evaluated in the 0.5–2.5 mL interval.

When the injected volume was lower than 1.0 mL, only transitional signals were observed (Fig. 3a and b). A well-defined plateau region (steady-state signals) for Na and Li was observed for volumes of L1 and L2 ≥ 1.5 mL (Fig. 3c and d). Although higher volumes decrease the sampling rate, the sampling loops corresponding to 2.5 mL were chosen for further studies since the main purpose here was illustrate the SDA in FIA system, not increase the sample throughput.

3.2. Optimization of SDA

A full signal record of one analytical cycle requires 6000 spectra, from which ca. 1000 spectra correspond to the SDA region. Considering the excessive number of spectra, the use of average spectra after 10–50 successive measurements was then evaluated. The variation in the number of average spectra (100–20) interval did not alter significantly the results. So, the use of 30 spectra to calculate an average spectrum was chosen for further studies.
The influence of selection of sets of points at different parts of the SDA region was evaluated at the following four sub-regions: 100–0% (all points, Fig. 4a), 90–10% (Fig. 4b), 90–0% (Fig. 4c) and 100–10% (Fig. 4d). These studies were carried out with 0.5 mg L\(^{-1}\) Na sample solution, deionized water (blank) and 1.0 mg L\(^{-1}\) Na + 1.0 mg L\(^{-1}\) Li (standard solution). Analysis of Fig. 4 reveals that better precision (typically 2%) and more accurate results for five samples were obtained for sub-regions comprised in the ranges of 90–10% (Fig. 4b) and 100–100% (Fig. 4d). The low emission intensities corresponding to final points were used (Fig. 5d), that was not observed (Y intercept: 0.9967, 0.8716, 0.8153 and 0.9591). When only the central points were used (Fig. 5b), showing those higher values have significance influence on trend line and Y intercept (0.8716, 0.8153 and 0.9591). When only the central points were used (Fig. 5d), that was not observed (Y intercept: 0.9967, 1.0139 and 1.0249). These findings suggest better results can be obtained when final points were eliminated. Thus, the sub-regions 90–10% or 100–10% can be employed for FIA-SDA calibration.

### 3.3. Sodium determination

After the FIA-SDA system optimization, accuracy was checked by analyzing five CRMs and the results for Na determination (Table 1) were in agreement with certified values at 95% confidence level (t-test). In addition, Na was also determined by using ES, IS and SA as comparative calibration methods. The results obtained for Na in biodiesel and whole milk powder using ES did not agree with certified values at 95% confidence level (t-test). The content of Na in biodiesel was ca. 2.3 times higher than the certified value. This may be attributed to the increase in flame temperature caused by ethanol and reduced surface tension (increasing the nebulization of the sample and also the atomic population in flame) [16,17]. Unsatisfactory result for Na in biodiesel was also observed by using IS. The underestimated recovery (76%) was probably caused by the different behavior of Na and Li concerning the increase in the flame temperature due to sample atomization. This effect can be related to different energy transitions relative to emission lines of Na (2.1044 eV) and Li (1.8478 eV) [18]. Results obtained for others CRMs were in agreement with the certified values at the 95% confidence level with the certified values. Regarding SA calibration, results for Na were in agreement with the certified values at the 95% confidence level (t-test). Considering the SA method requires a series of standards, the proposed method needs only two solutions per analysis. The RSD calculated for the FIA-SDA and SA were 4.4% and 7.3%, respectively.

The FIA-SDA system was also applied to the determination of Na in commercial biodiesel samples (Table 2). For comparison purposes, samples were also analyzed by using ES, IS and SA. Analysis of Table 2 reveals that only results for Na determination by the SA method were in agreement at the 95% confidence level (paired t-test) with the results obtained by FIA-SDA. The RSD (n = 3) calculated for samples were in the 1.3–9.3% (FIA-SDA) and 3.3–16.9% (SA) intervals.

The performance of the FIA-SDA system was also evaluated by addition and recovery tests in all samples (Table 3). Recoveries of Na were in the following ranges: 181–202% (ES), 67–72% (IS), 111–126% (SA), and 94–98% (FIA-SDA). These findings reinforce the efficacy of the proposed system described in this work.

### 4. Conclusions

It was demonstrated here the possibility of using SDA in a flow system. The proposed method combines the benefits of two classic methods (standard additions and internal standardization) with the convenience of preparing only two solutions per sample. The efficiency of SDA in flow system was demonstrated here for the determination of Na in biodiesel samples by atomic emission spectrometry, but it can be adapted to various analytes, samples and techniques. SDA requires the simultaneous measurement of both analyte and internal standard. The optical fiber probe coupled to flame AAS was employed in this work due to the unavailability of a simultaneous spectrometer in our laboratory. However, the FIA-SDA method can obviously be carried out in any commercial simultaneous instrument.

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